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EFFECT OF EDARAVONE IN ISONIAZID-INDUCED HEPATOTOXICITY IN SPRAGUE DAWLEY RATS

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ABSTRACT

Isoniazid is a synthetic derivative of nicotinic acid with anti-mycobacterial properties. It is commonly used for the treatment of tuberculosis. The mechanism of liver damage induced by isoniazid appears to be due to metabolic idiosyncratic reactions. The progression of hepatotoxicity mainly involves the formation of toxic intermediaries and reactive oxygen series. Edaravone is a potent anti-oxidant. It strongly scavenges free radicals, protecting the cells against oxidative stress. Due to the contribution of oxidative stress to the pathogenesis of hepatotoxicity, we tested the hypothesis of edaravone in hepatotoxicity and give protection against biochemical signs of isoniazid hepatotoxicity. Various parameters including animal body weight, liver weight, serum total bilirubin, serum aspartate aminotransferase, alanine aminotransferase and serum alkaline phosphatase were analysed in hepatotoxicity animals (induced with isoniazid 54 mg/kg/body weight *p.o.*) with edaravone (10 mg/kg/body weight *i.p.*) and silymarin (10 mg/kg/body weight *p.o.*). Isoniazid control rats demonstrated that no changes in the body weight, while liver weight, serum total bilirubin, serum aspartate aminotransferase, alanine aminotransferase and serum alkaline phosphatase were increased significantly. Edaravone attenuated all the biochemical analyses such as, serum total bilirubin, serum aspartate aminotransferase, alanine aminotransferase and serum alkaline phosphatase. Moreover the liver weight also reduced but not statistically significantly while compared with isoniazid control rats. The standard drug silymarin reduces all biochemical parameters significantly. Our study suggests that edaravone prevented liver structural and functional abnormalities associated with experimental hepatotoxicity. With all the biochemical parameters we concluded that edaravone reduced the toxicity caused by isoniazid and can be used against hepatotoxicity.

Keywords: Isoniazid, Edaravone, Hepatotoxicity, Hepatoprotection

INTRODUCTION

The failure of liver due to overloaded of medicines or xenobiotic is known as hepatotoxicity. Not only chemicals but also many of the drugs which are in clinical exercise are considered as hepatotoxic agents. However, the drugs are concerned with hepatic injury, including regularly used analgesics, paracetamol, frequently molested alcoholic beverages and first-line anti-tubercular agents. Hepatotoxicity encouraged by a drug not only limits their further use but it also might inhibit with needed metabolic roles [1].

The most corporate way to disruption of tuberculosis chemotherapy is owed to the development of drug-induced hepatotoxicity during the chemotherapy period. Wide variations have been detected in the hepatotoxic reactions incidences through short course chemotherapy from diverse countries with the reported frequency being 3% in USA, 4% in UK, 9.9% in Argentina, 11% in Germany, 13% in Hong Kong, 26% in Taiwan, 8%–36% in India and 36% in Japan [2]. Currently, the anti-tubercular chemotherapeutic treatments contain isoniazid cause potentially severe adverse effects, prominent to liver toxicity initiated due to drug-induced hepatotoxicity.

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Isoniazid is a synthetic derivative of nicotinic acid with anti-mycobacterial properties. It is regularly used for the treatment and prophylaxis of tuberculosis. It blocks the synthesis of mycolic acids, main components of the mycobacterial cell wall, thus killing *Mycobacterium tuberculosis* organisms. Subsequently in 1965 American Thoracic Society guidelines, the first-line option of Isoniazid in treating latent *Mycobacterium tuberculosis* infection due to its significant effect in reducing morbidity especially in high threat people [3]. However, Isoniazid-induced hepatotoxicity is a main alarm for clinical treatment due to the concomitant use of numerous medicines and long treatment period [4].

Edaravone is a synthetic-free radical scavenger and chemically known as 3-methyl-1-phenyl-2-pyrazolin-5-one [5]. Japan introduced in worldwide as a first neuroprotective drug in treating patients with cerebral infarction [5]. Moreover it's has a strong free radical scavenging properties, edaravone possesses anti-apoptotic and anti-necrotic effects in varies diseases of animal models [6]. Therefore, it might be essential to examine its therapeutic potential in hepatotoxicity in associated with oxidative stress and cell death. Several studies results confirmed that edaravone has beneficial actions on renal and cardiovascular. Moreover edaravone reduced the

oxidative stress and upgraded acute myocardial infarction by the long-term therapy [7-9]. In result, edaravone was proposed to be a new choice for the management of cardiovascular disease [6]. Remarkably, edaravone has been shown to safeguard canine kidneys from ischemia-reperfusion injury by improving the renal vascular resistance, tubular cell function, and dropping the mean serum creatinine [10]. The present study aimed to investigate the effect of edaravone in liver toxicity (hepatotoxicity) in rats.

METHODS

Drugs and chemicals:

Edaravone and Silymarin were purchased from Sigma-Aldrich Ltd., St. Louis, MO, USA. Isoniazid was purchased from R & M Marketing, Esser, U.K. All other chemicals used in the present study were of analytical grade. Isoniazid (54 mg/kg body weight *p.o.)* dissolved in freshly prepared saline solution was administered to induce hepatotoxicity in rats. Edaravone (10 mg/kg/day, *i.p.*) and silymarin (10 mg/kg/body weight *p.o.*) were dissolved in freshly prepared saline solution was administered to edaravone pre-treatment group and silymarin pre-treatment group. At the end of the study, the alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and bilirubin were measured.

Animals:

This study was carried out for 14 days. All the experimental procedures were approved by the AIMST University Human and Animals Ethics Committee (AUHAEC 19/FOP/2017) and complied with the guidelines of the care and use of laboratory animals at the AIMST University, Malaysia. Rats were acclimatized in the AIMST Central Animal House and allowed for access of water and food ad libitum. Rats were exposed to normal day and night cycles.

Experimental protocol:

Twenty four healthy male Sprague Dawley (SD) rats, weighing between 170-200 gm were used in the study. Four groups were employed in the study and each group comprised six rats.

Group I: Normal control group: Normal control (NC) rats were kept, maintained and allowed free access to water and food. No drug or treatment was given. 0.9% normal saline was administered daily once *i.p*., according to their body weight.

Group II: Isoniazid control group**:** Isoniazid (INH) was administered to rats at a dose of 54 mg/kg/body weight *p.o*. daily once for the induction of hepatotoxicity.

Group III: Isoniazid + Edaravone (pre-treated) group: The rats were given edaravone (EDA) via intraperitoneal route at a dose of 10 mg/kg/body weight daily once and isoniazid was given at a dose of 54 mg/kg/body weight *p.o*. daily after one hour of pre-treated edaravone.

Group IV : Isoniazid + Silymarin (pre-treated) group: The rats were given silymarin (SILY) via oral route at a dose of 10 mg/kg/body weight daily once and isoniazid was given at dose of 54 mg/kg/body weight *p.o*. daily after one hour of pre-treated silymarin.

Induction of experimental hepatotoxicity: Rats administered with isoniazid (54 mg/kg/day *p.o.*) were allowed for 14 days to develop experimental hepatotoxicity.

Estimation of aspartate aminotransferase (AST):

The serum aspartate aminotransferase concentration was estimated using commercially available Reflotron strips employing Reflotron Plus Apparatus (Roche Diagnostics, Germany). The principle involved in the estimation of serum aspartate aminotransferase is shown below. After application to the test strip, the sample flows into the reaction zone. In the presence of GOT, α-ketoglutarate and alanine sulfonate are converted to pyruvate and glutamate. In further reactions steps, hydrogen peroxide is formed that reacts with the indicator to form a blue dye, which is directly proportional to the serum aspartate aminotransferase present in the sample;

$$\alpha\text{-ketoglutarate} + \text{Alanine} \xrightarrow{\text{GOT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + PO_4^{3-} + O_2 + H_2O \rightarrow \text{Acetyl phosphate} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{indicator} \rightarrow \text{Dye} + H_2O$$

Scheme 1: Aspartate aminotransferase reaction

At a temperature of 37°C, the dye formed was measured at 567nm, and the serum aspartate aminotransferase was expressed in U/I.

Estimation of alanine transaminase (ALT):

The serum alanine transaminase concentration was estimated using commercially available Reflotron strips employing Reflotron Plus Apparatus (Roche Diagnostics, Germany). The principle involved in the estimation of serum alanine transaminase is shown below. After application to the test strip, the sample flows into the reaction zone. In the presence of GPT, α- ketoglutarate and alanine are converted to glutamate and pyruvate. In the second reaction step, catalyzed by pyruvate oxidase, the resulting pyruvate is cleaved into acetyl phosphate, carbon dioxide and hydrogen peroxide. Finally by the presence of POD the hydrogen peroxide converts an indicator into its oxidized blue form:

$$\alpha\text{-ketoglutarate} + \text{Alanine} \xrightarrow{\text{GPT}} \text{glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + PO_4^{3-} + O_2 + H_2O \xrightarrow{\text{PyOD}} \text{Acetyl phosphate} + H_2O_2 + CO_2$$

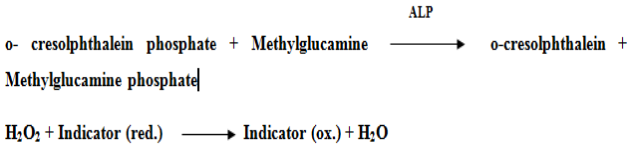
$$H_2O_2 + \text{Indicator (red.)} \xrightarrow{\text{POD}} \text{Indicator (ox.)} + H_2O$$

Scheme 2: Alanine transaminase reaction

At a temperature of 37°C, the dye formed was measured at 567nm, and the serum alanine transaminase was expressed in U/I.

Estimation of alkaline phosphatase (ALP):

The serum alkaline phosphatase concentration was estimated using commercially available Reflotron strips employing Reflotron Plus Apparatus (Roche Diagnostics, Germany). The principle involved in the estimation of serum alkaline phosphatase follows. After application to the test strip, the sample flows into the reaction zone. ALP hydrolyzes o- cresolphthalein phosphate to o-cresolphthalein and transfers the phosphate group to the acceptor molecule methylglucamine. The colored hydrolysis product o-cresolphthalein is produced per unit of time under alkaline conditions is directly proportional to alkaline phosphatase activity:

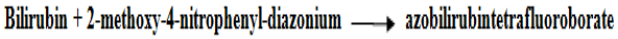


Scheme 3: Alkaline phosphatase reaction

At a temperature of 37°C, the dye formed was measured at 567nm, and the serum alkaline phosphatase was expressed in U/I.

Estimation of total bilirubin:

After application to the test strip, the sample flows into the reaction zone, where, in the case of blood samples, the separation of the erythrocytes from the plasma occurs. Before the beginning of the reaction, the protein-bound indirect bilirubin is released by means of dyphiline [7-(2,3-dihydroxyprophyl)-theophylline]. Both the direct and the indirect bilirubin react with the diazonium salt 2-methoxy-4-nitrophenyldiazoniumtetrafluoroborate.



Scheme 4: Total bilirubin reaction

At a temperature of 37°C, the bilirubin concentration (proportional to the dye formed) was measured at a wavelength of 567 nm, and the serum bilirubin was expressed in mg/dL or µmol/L.

Biochemical assessments of hepatotoxicity:

The development of hepatotoxicity was assessed in rats by measuring the mean body weight and liver weight, serum aspartate aminotransferase, alanine aminotransferase and serum alkaline phosphatase and serum total bilirubin level. The biochemical estimations were done using commercially available Reflotron strips assay kit employing Reflotron Plus Apparatus (Roche Diagnostics, Germany).

At the end of 14 days study, the treated and untreated rats were euthanized and sample was collected by cardiac puncture. Blood sample from each rat was collected, centrifuged at 9000 rpm for 5 min and serum was collected. Using a micropipette, 30 µL of serum was drawn into the pipette (avoiding bubbles) and applied as a drop to the centre of the red application zone on the Reflotron assay strips, without touching the application zone. Before starting the analysis, a calibration for each parameter was done using standard calibration strips. The calibration value was in the range of (631~ 651), (632~652) and (624~644) nm. The assay strip was placed into the Reflotron apparatus for biochemical analysis. The concentrations of serum aspartate aminotransferase, serum alanine aminotransferase, serum alkaline phosphatase and serum total bilirubin level were expressed in U/I and mg/dL simultaneously.

Statistical analysis:

The results were expressed as mean ± standard error mean (SEM). Data obtained from various groups were statistically analysed by one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The '*p*' value of less than 0.05 was considered as statistically significant.

RESULTS

Mean body weight:

The mean body weight of normal control group, INH control group (54 mg/kg/body weight. *p.o.),* edaravone treated group (10 mg/kg/body weight. *i.p.*) and silymarin treated group (10 mg/kg/body weight. *p.o.*) are shown in the figure 1. The mean body weight of normal control group, INH control group, edaravone treated group and silymarin treated group did not show any statistically significant among the four groups.

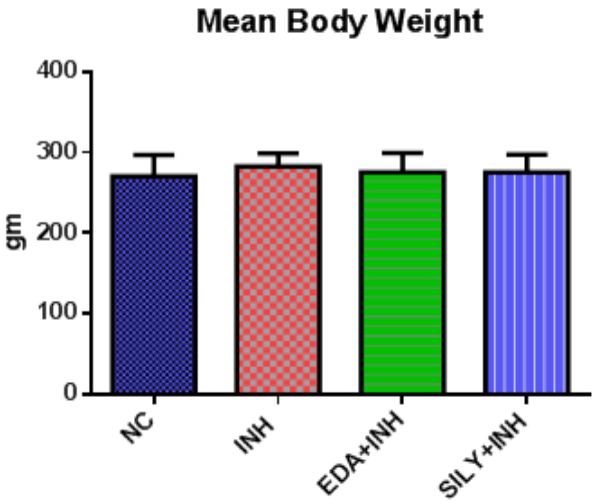


Figure-1: Effect of edaravone and silymarin on mean body weight (gm) on 14 days study. Data are expressed as mean ± SEM. Body weight did not statistically differ among the four groups (n=6).

Mean liver weight:

The mean liver weight of animals in normal control group, INH control group, edaravone treated group and silymarin treated group are shown in the figure 2. As compared to the normal control animals, the INH control group showed a slight increase in mean liver weight, this increase was statistically significant. In contrast, there was a decrease in the mean liver weight in edaravone

treated and silymarin treated group when compared to INH control group, but this mean liver weight was not statistically significant.

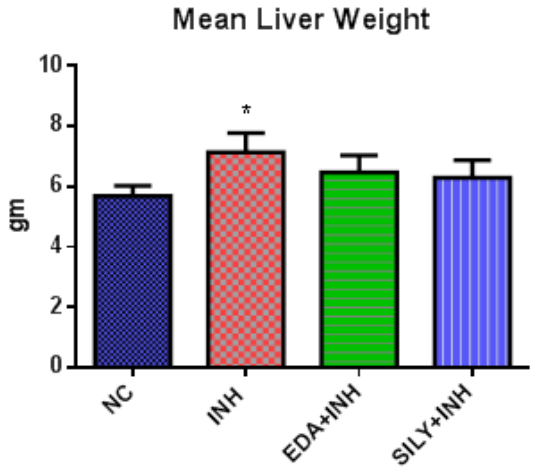


Figure-2: Effect of edaravone and silymarin on mean liver weight (gm). Data are expressed as mean ± SEM. *P< 0.05 vs Normal control.

Serum total bilirubin:

The serum total bilirubin of animals in normal control group, INH control group, edaravone treated group and silymarin treated group are shown in Figure 3.3. The serum total bilirubin level in INH control group rats increased significantly (*P*< 0.001) as compared to normal control group. However, INH rats treated with either edaravone or silymarin showed a significant (*P*< 0.05) reduction in the serum total bilirubin level as compared to the INH control group (Figure 3).

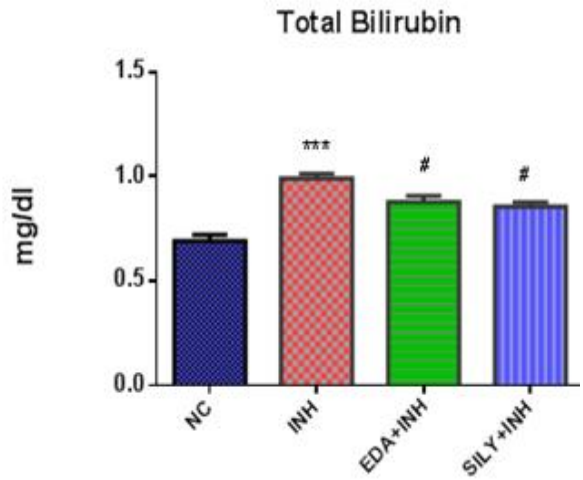


Figure-3: Effect of edaravone and silymarin on serum total bilirubin (mg/dL) concentration. Data are expressed as mean ± SEM. ***P< 0.001 vs Normal control; #P< 0.05 vs INH control.

Aspartate aminotransferase (AST):

The serum aspartate aminotransferase was noted to be significantly increased in INH control group as compared to normal control rats (*P*<0.05). Interestingly, edaravone treatment group and silymarin treatment group showed an observable decrease in serum aspartate aminotransferase level as compared to the INH control group (*P*< 0.05) (Figure 4).

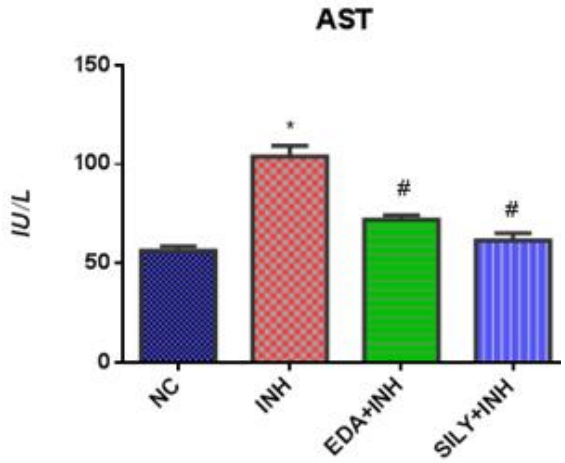


Figure-4: Effect of edaravone and silymarin on serum aspartate aminotransferase (*IU/L*) concentration. Data are expressed as mean ± SEM. *P< 0.05 vs Normal control; #P< 0.05 vs INH control.

Alanine aminotransferase (ALT):

The serum alanine aminotransferase was noted to be significantly increased in INH control group as compared to normal control rats (*P*<0.01). Interestingly, edaravone treatment group and silymarin treatment group showed an observable decrease in serum alanine aminotransferase level as compared to the INH control group (*P*< 0.05) (Figure 5).

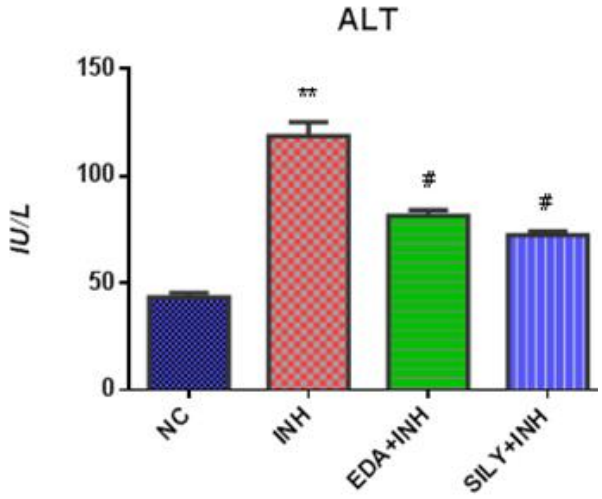


Figure-5: Effect of edaravone and silymarin on alanine aminotransferase (*IU/L*) concentration. Data are expressed as mean ± SEM. **P< 0.01 vs Normal control; #P< 0.05 vs INH control.

Serum alkaline phosphatase (ALP):

The serum alkaline phosphatase of animals in normal control group, INH control group, edaravone treated group and silymarin treated group are shown in figure 3.6. The serum alkaline phosphatase level in INH control group rats increased significantly (*P*< 0.01) as compared to normal control group. However, INH rats treated with either edaravone or silymarin showed a significant (*P*<

0.05 and *P*< 0.01) reduction in the serum alkaline phosphatase level as compared to the INH control group (Figure 6).

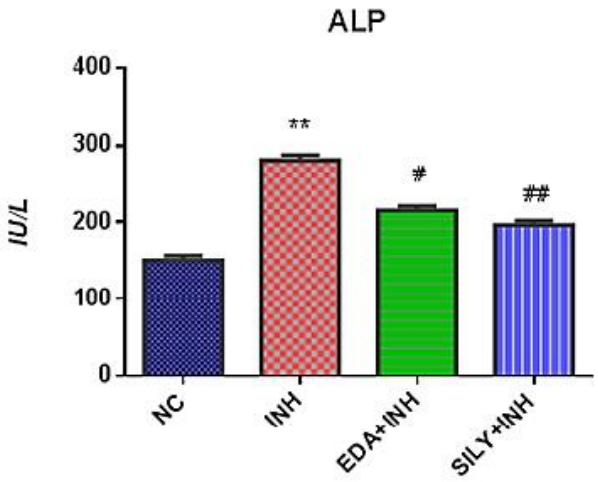


Figure-6: Effect of edaravone and silymarin on serum alkaline phosphatase (*IU/L*) concentration. Data are expressed as mean ± SEM. $^{**}P$< 0.01 vs Normal control; $^{\#}P$< 0.05; $^{\#\#}P$< 0.01vs INH control

DISCUSSION

The present results revealed that the administration of edaravone (10 mg / kg of body weight *i.p.*) and silymarin (10 mg / kg of body weight *p.o.*) ameliorates the isoniazid (54 mg / kg of body weight *p.o.*) induced hepatic failure. The isoniazid induced hepatotoxicity has been indicated by increasing of liver weight, total bilirubin, AST, ALT and ALP. These effects are attenuates by edaravone treatments. Whereas, the changes of isoniazid induced liver weight has not altered by edaravone treatment. The similar results has been observed in reference compound *i.e.,* silymarin treatment.

Isoniazid is widely used for the treatment of *Mycobacterium tuberculosis* infection [11]. It is first line drug for tuberculosis infection. However, the chronic treatment of tuberculosis with isoniazid is alters the hepatic metabolism. The hydrazide from of INH is readily oxidized with hepatic metabolic enzymes and produced the four bioactive metabolites i.e., isonicotinic acid, acetyl hydrazine (AcHz), hydrazine (Hz), and ammonia. The AcHz and Hz are plays a key role to induction of hepatic injury and failure of liver tissue function. The bio-reactive metabolites are known to produce the strong covalent bond with hepatic macromolecules. This covalent binding of INH toxic metabolites are accelerates the hepatic immune cell responses via acetylation of cellular immune response proteins [12]. Further, INH adducts are also interacts with D-dopachrome decarboxylase, prohibitin-2 and macrophage migration inhibitory factor [13]. In addition to that, INH metabolites are also involved in the generation of free radicals via rising of mitochondrial oxidative stress and dysfunction [14]. The multiple experimental results are revealed that, INH induced the hepatotoxicity with indication of bilurupin, AST, ALT and ALP. Furthermore, it also alters the liver weight with interaction of multiple immunological and hepatic macromolecular proteins like hepatic growth factors [15]. The same results have been observed in the present study. Whereas, the treatment of edaravone has been attenuate the INH induced above hepatic toxic changes.

Edaravone has potent free radical scavenger and immune-regulatory mechanism. It is widely, used for the treatment of a stroke and amyotrophic lateral sclerosis. The effect of edaravone reduces the cellular oxidative stress via interaction and regulation of hepatic oxidative defense enzymes [16-17]. Furthermore, it protects the mitochondria from various cellular insults like free radicals, immune reaction, hypoxia, ischemia and toxic metabolites [18-20]. However, this is a first report of edaravone for hepato-protective effect against the INH induced hepatic failure. The hepatoprotective effect of edaravone has been confirmed by estimation of multiple hepato specific biomarkers like bilirubin, AST, ALT and ALP. The results are also supported with known hepatoprotective agents *i.e.,* silymarin. Silymarin is also one of the strong antioxidant agents. The hepatoprotective effects of silymarin also possess the regulation of mitochondria protein, immunological proteins and liver regenerative proteins [21- 22]. In the present study, it attenuates the INH induced changes of hepatic biomarkers like bilirubin, AST, ALT and ALP. Therefore, edaravone can be used for the hepatic failure disorders.

CONCLUSION

Hence, it may be concluded that edaravone may be useful medicine for the treatment of drug induced hepatotoxicity especially; INH induced hepatic failure due to its potential anti-oxidant, anti-inflammatory, immunoregulatory, hepatic regenerative and mitochondrial protective actions. However, the more extensive studies are required to explore the multiple molecular mechanisms in different pathophysiological conditions of liver.

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